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Mapping the major interaction between binding protein and Ig light chains to sites within the variable domain.

Davis DP, Khurana R, Meredith S, Stevens FJ, Argon Y.

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Department of Pathology, University of Chicago, IL 60637, USA.

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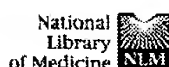
Newly synthesized Ig chains are known to interact in vivo with the binding protein (BiP), a major peptide-binding chaperone in the endoplasmic reticulum. The predominant interactions between the light chain and BiP are observed early in the folding pathway, when the light chain is either completely reduced, or has only one disulfide bond. In this study, we describe the *in vitro* reconstitution of BiP binding to the variable domain of light chains (VL). Binding of deliberately unfolded VL was dramatically more avid than that of folded VL, mimicking the interaction in vivo. Furthermore, VL binding was inhibited by addition of ATP, was competed with excess unlabeled VL, and was demonstrated with several different VL proteins. Using this assay, peptides derived from the VL sequence were tested experimentally for their ability to bind BiP. Four peptides from both beta sheets of VL were shown to bind BiP specifically, two with significantly higher affinity. As few as these two peptide sites, one from each beta sheet of VL, are sufficient to explain the association of BiP with the entire light chain. These results suggest how BiP directs the folding of Ig in vivo and how it may be used in shaping the B cell repertoire.

PMID: 10490983 [PubMed - indexed for MEDLINE]

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☐ 1: EMBO J 1992 Apr;11(4):1573-81

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Interaction of BiP with newly synthesized immunoglobulin light chain molecules: cycles of sequential binding and release.

Knittler MR, Haas IG.

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Institut für Genetik, Universität zu Köln, Germany.

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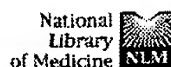
Here we show that not only transport defective but all immunoglobulin light chains interact with BiP. Association of BiP with its ligand takes place during or shortly after translation of the light chains. The biological half life of the BiP-light chain complex depends on the fate of the light chains. Light chains which are secreted interact with BiP for only a very short time. In contrast, the complex is biologically more stable in cells which do not secrete their L chains. In these cells, dissociation from BiP correlates with the biological half life of the L chains arguing for a degradation pathway in the endoplasmic reticulum. Instead of being degraded in association with its ligand, BiP is released from the complex and binds to newly synthesized polypeptides. These results support the notion that both H and L chains require the chaperoning function of BiP before or during the process of antibody assembly.

PMID: 1563355 [PubMed - indexed for MEDLINE]

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28;92(5):1764-8

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Molecular chaperones involved in protein degradation in the endoplasmic reticulum: quantitative interaction of the heat shock cognate protein BiP with partially folded immunoglobulin light chains that are degraded in the endoplasmic reticulum.

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Knittler MR, Dirks S, Haas IG.

Institute for Biochemistry I, University of Heidelberg, Germany.

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In the absence of immunoglobulin heavy-chain expression, some immunoglobulin light (L) chains are retained and degraded within the cell. We investigated the fate of two different nonsecreted murine L chains which exhibit different half-lives (50 min and 3-4 hr). Our results demonstrate that both nonsecreted L chains are quantitatively bound to BiP as partially oxidized molecules. The kinetics of L-chain degradation coincided with those of L-chain dissociation from BiP, which suggests that these two processes are functionally related. L-chain degradation does not depend on vesicular transport, indicating that these soluble proteins are degraded in the endoplasmic reticulum (ER). In contrast, secreted L chains, which interact only transiently with BiP, are completely oxidized and are not degraded even when they are artificially retained in the ER. Our data support the model that, by means of BiP interaction, the ER degradation mechanism has the potential to discriminate between partially and completely folded molecules.

PMID: 7878056 [PubMed - indexed for MEDLINE]

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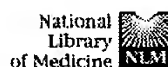
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☐ 1: Immunity 2000 Oct;13(4):433-42

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Inhibition of amyloid fiber assembly by both BiP and its target peptide.

Davis PD, Raffen R, Dul LJ, Vogen MS, Williamson KE, Stevens JF, Argon Y.

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Department of Pathology and Committee on Immunology, The University of Chicago, Illinois 60637, USA.

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Immunoglobulin light chain (LC) normally is a soluble, secreted protein, but some LC assemble into ordered fibrils whose deposition in tissues results in amyloidosis and organ failure. Here we reconstitute fibril formation in vitro and show that preformed fibrils can nucleate polymerization of soluble LC. This prion-like behavior has important physiological implications, since somatic mutations generate multiple related LC sequences. Furthermore, we demonstrate that fibril formation in vitro and aggregation of whole LC within cells are inhibited by BiP and by a synthetic peptide that is identical to a major LC binding site for BiP. We propose that LC form fibrils via an interprotein loop swap and that the underlying conformational change should be amenable to drug therapy.

PMID: 11070162 [PubMed - indexed for MEDLINE]

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